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L2: Entry 1 of 1 File: USPT Feb 9, 1999

DOCUMENT-IDENTIFIER: US 5869445 A
TITLE: Methods for eliciting or enhancing reactivity to HER-2/neu protein

US Patent No. (1):
5869445

Detailed Description Text (32):
Preferred yeast vectors can be assembled using DNA sequences from pBR322 for selection and replication in E. coli (Amp.sup.r gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and .alpha.-factor secretion leader. The ADH2 promoter has been described by Russell et al. (J. Biol. Chem. 258:2674, 1982) and Beier et al. (Nature 300:724, 1982). The yeast .alpha.-factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed (see, e.g., Kurjan et al., Cell 30:933, 1982; and Bitter et al., Proc. Natl. Acad. Sci. USA 81:5330, 1984). The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes. The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from polyoma, adenovirus 2, simian virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the Hind III site toward the Bgl II site located in the viral origin of replication is included. Further, viral genomic promoter, control and/or signal sequences may be utilized, provided such control sequences are compatible with the host cell chosen. Exemplary vectors can be constructed as disclosed by Okayama and Berg, Mol. Cell. Biol. 3:280, 1983.

Detailed Description Text (72):
The human HER-2/neu polypeptide was recovered by the PCR method (e.g., U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159) from a plasmid prepared according to Di Fiore et al. (King et al., Science 229:974-976, 1985; Di Fiore et al., Science 237:178-182, 1987) using oligonucleotide primers that additionally introduced a BssHII restriction site and an enterokinase protease site on the 5' end and an EcoRI site on the 3' end. The primer for the 5'-end was 5'-TCTGGCGCGCTGGATGACGATGACAAGAAACGACGGCAGCAGAAGATC-3' (SEQ ID NO:3) while the primer for the 3'-end was 5'-TGAATTCTCGAGTCATTACACTGGCACGTCCAGACCCAG-3' (SEQ ID NO:4). The resulting 1.8 kb PCR fragment was subcloned into the T-vector from Novagen (Madison, Wis., USA) and the sequence of selected clones was determined on the ABI 373 automated DNA sequencer (Applied Biosystems Inc., Foster City, Calif., USA) using overlapping sequencing primers. PCR fragments with sequence that corresponded to the published DNA sequence for the human HER-2/neu cDNA (SEQ ID NO:1; Coussens et al., Science 230:1132, 1985; Yamamoto et al., Nature 319:230, 1986)were then connected in the correct reading frame via the BssHII site to a modified E. coli thioredoxin reductase. A 6Xhistidine affinity tag employed in Ni-NTA affinity purification of the expressed fusion protein was incorporated into the thioredoxin reductase fusion partner. This cDNA for the trxA-human HER-2/neu polypeptide fusion protein was subcloned into a modified pET expression vector for expression in E. coli.

Detailed Description Text (73):
While thioredoxin reductase has been reported to stabilize and solubilize other heterologous proteins expressed in E. coli, it did not appear to offer any significant advantage for human HER-2/neu polypeptide expression in E. coli. While a significant proportion of the trxA-HER-2/neu polypeptide fusion protein was soluble, a majority was expressed in inclusion bodies. The fusion protein was also subjected to degradation during expression in E. col. The presence of the thioredoxin reductase fusion partner may, however, stabilize the protein during purification. The availability of monoclonal antibodies to thioredoxin reductase provides a convenient marker to follow during purification.

Detailed Description Text (74):
For purification of the human HER-2/neu polypeptide with the thioredoxin reductase fusion partner containing the 6XHis affinity tag, the E. coli pellet was resuspended with protease inhibitors and lysozyme and sonicated. The inclusion bodies were isolated by centrifugation, and are washed 3X with deoxycholate, the last wash being overnight to remove LPS. The washed inclusion bodies are solubilized in GuHCl for Ni purification. The Ni column was eluted with Imidazole in urea and dialyzed against 10 mM Tris pH8. The recovery of HER-2/neu polypeptide using this protocol was from 80%-95% pure full length protein with the main contaminant being degraded protein. From 500 ml of fermentation, 20 mg were recovered. It was >98% HER-2/neu polypeptide. The techniques used herein are well known to those in the art and have been described, for example, in J. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1989, Cold Spring Harbor, N.Y., USA.

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TITLE: Methods for eliciting or enhancing reactivity to HER-2/neu protein

US Patent No. (1):
5869445

Brief Summary Text (12):
In one aspect, the present invention provides compounds and compositions that elicit or enhance an immune response to HER-2/neu protein. One embodiment of the present invention provides a polypeptide encoded by a DNA sequence selected from: (a) nucleotides 2026 through 3765 of SEQ ID NO:1; and (b) DNA sequences that hybridize to a nucleotide sequence complementary to nucleotides 2026 through 3765 of SEQ ID NO:1 under moderately stringent conditions, wherein the DNA sequence encodes a polypeptide that produces an immune response to HER-2/neu protein. In a preferred embodiment, a polypeptide has the amino acid sequence of SEQ ID NO:2 from lysine, amino acid 676, through valine, amino acid 1255, or a variant thereof that produces at least an equivalent immune response. A composition is provided that comprises a polypeptide of the present invention in combination with a pharmaceutically acceptable carrier or diluent. In another embodiment, a nucleic acid molecule directing the expression of a polypeptide according to the present invention is provided. In another embodiment, a viral vector directing the expression of a polypeptide according to the present invention is provided.

Detailed Description Text (3):
HER-2/neu polypeptide-as used herein, refers to a portion of the HER-2/neu protein (the protein also known as p185 or c-erbB2) having the amino acid sequence of SEQ ID NO:2 from lysine, amino acid 676, through valine, amino acid 1255; and may be naturally derived, synthetically produced, genetically engineered, or a functionally equivalent variant thereof, e.g., where one or more amino acids are replaced by other amino acid(s) or non-amino acid(s) which do not substantially affect elicitation or enhancement of an immune response to HER2/neu protein (e.g., variant stimulates a response by helper T cells or cytotoxic T cells).

Detailed Description Text (18):
Variants within the scope of this invention may also, or alternatively, contain other modifications, including the deletion or addition of amino acids, that have minimal influence on the desired immunological properties of the polypeptide. It will be appreciated by those of ordinary skill in the art that truncated forms or non-native extended forms of a HER-2/neu polypeptide may be used, provided the desired immunological properties are at least roughly equivalent to that of full length, native HER-2/neu polypeptide. Cysteine residues may be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present.